### **REMARKS**

Applicants respectfully request reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claims 1-15 are currently pending and claims 1-4, 9-13 and 15 are amended.

Applicants acknowledge that the Examiner has agreed to examine SEQ ID NOS: 12, 60 and 113 together.

Applicants confirm the election of Group I with traverse. Further, applicants point out that rejoinder of process of using the product claims of Group I commensurate in scope with the product claims determined as allowable is permitted under the *Ochiai* guidelines.

Applicants request rejoinder of claims 12-14, Group II, and claim 15, Group III, upon allowance of the claims of Group I. Applicants have amended the claims so that the polynucleotide sequences and genetic constructs contain the same sequences from SEQ ID NO: 113.

The amendments to the claims have support in the specification and claims as filed. The amendment to claim 1 can be found throughout the specification and specifically, in the description of Figure 2 on page 5, lines 14-15, which recites that Figure 2 discloses the nucleotide sequence of *Eucalyptus grandis* cOMT gene and promoter. Page 6, lines 14-16 and page 7, beginning on line 3 disclose that the isolated polynucleotide sequence of the present invention has vascular tissue-specific promoter activity of the *E. grandis* cOMT gene. The amendments to claims 2, 4 and 9 are supported specifically in original claim 3, the description of Figures 3 and 4 on page 5, beginning at line 16 to page 6, line 5, page 3, lines 12-18. Support for amending claims 10-12 to also depend from claim 9 on page 15 lines 10-16. Claim 15 is amended to also include a *E. grandis* cOMT promoter of SEQ ID NO: 113.

### I. Priority

The Examiner acknowledges that the present application is a CIP of a number of referenced patent applications. The examiner states that SEQ ID NOS: 12, 60 and 113 are first disclosed in U.S. Serial No. 09/724,624, that was filed on November 28, 2000, and therefore, that this date is the priority date of the claims directed to those sequences in the present application.

Applicants respectfully disagree with the Examiner's analysis and, in fact, submit that SEQ ID NO: 12 was first disclosed in U.S. Serial No.: 09/276,599, now U.S. Patent 6,380,459 filed on March 25, 1999, as SEQ ID NO: 12 in this latter application. Further, SEQ ID NOS: 60 and 113 were first disclosed in U. S. Serial No.: 09/598,401, now U.S. Patent 6,596,925, filed June 20, 2000, and then again in U.S. Serial No.: 09/724,624 filed November 28, 2000, now abandoned. Additionally, the provisional patent application, U.S. Serial No.: 60/146,591, filed on July 30, 1999, which is in the priority chain of U.S. Patent 6,596,925, discloses SEQ ID NO: 60. Therefore, applicants submit that SEQ ID NO: 12 has a priority date of March 25, 1999, SEQ ID NO: 60 has a priority date of July 30, 1999 and SEQ ID NO: 113 has a priority date of June 20, 2000. Applicants have not provided copies of these patents and patent applications as the Examiner is able to readily obtain copies of these documents to verify applicants' statements. Applicants request that the Examiner review these documents to verify the correctness of applicants' statements and revise her priority statement, accordingly.

### II. Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-11 are rejected under 35 U.S.C.§ 112, first paragraph, as allegedly failing to comply with the written description requirement because the claims contains subject matter which was allegedly not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. In support of her position, the Examiner states that she must determine whether a representative number of species have been described by their complete structure and whether a representative number of species have been sufficiently described by other relevant identifying characteristics. The Examiner states that claims 1, 3, 9 and 11 recite a functional vascular tissue specific *E. grandis* cOMT promoter, and further states that "it is well known in the art that [a] promoter, in other words, the transcription regulatory element of a specific gene may be 5' or 3' UTR, or within the intronic region of the gene, or in sequences that are distal to the gene." Thus, the Examiner states that the specification only discloses that the sequences from the 5' UTR of the cOMT gene, particularly SEQ ID NO: 113 and one fragment SEQ ID NO: 12, have the vascular tissue

specific regulatory function, and therefore, does not disclose promoter sequences from other regions of the *E. grandis* cOMT gene that has the vascular tissue specific regulatory function.

To clarify the present invention, applicants have amended claims 1 and 9 to recite that the claimed polynucleotide sequence comprises "a functional vascular tissue-specific *E. grandis* cOMT promoter contained in SEQ ID NO: 113." This sequence is provided in Figure 2 and in the sequence listing. Further, the description of Figure 2 on page 5, lines 14-15, defines the promoter contained in SEQ ID NO: 113 as nucleotides 1019-1643, which are bolded in the sequence. So by defining that the claimed *E. grandis* cOMT promoter is a promoter sequence contained in SEQ ID NO: 113, applicant has provided the skilled person in the art with the sequence from which the promoters of the present invention are to be identified, isolated and analyzed. In addition to identifying SEQ ID NO: 12, which is 661 nucleotides in length, and 98.1% identical to the promoter comprising nucleotides 1019-1676 of SEQ ID NO: 113, (see page 7, lines 15-19) and nucleotides 1019-1643 as the cOMT promoter of SEQ ID NO: 113, the specification also discloses that the 5'UTR region of SEQ ID NO: 113 (nucleotides 1-1643) has promoter activity as shown in Figures 3 and 4 and Example 3. As noted by the Examiner, SEQ ID NO: 12 shows vascular tissue-specific expression of a DNA sequence (see Figure 5 and Example 3).

In addition to this data showing promoter activity of sequences from SEQ ID NO: 113 in the present application, applicants direct the Examiner's attention to co-pending U.S. Serial No. 10/703,091. See Attachment A for selected pages of this application. This application contains attached Table 1 in Example 2, on pages 39 and 40 of the specification, and discloses 7 fragments of *E. grandis* cOMT promoter of varying lengths, designated as SEQ ID NOS: 1-7. SEQ ID NO: 1 is 534 nucleotides in length and is 100% identical to nucleotides 1110-1643 of SEQ ID NO: 113. SEQ ID NOS: 1-5 were tested for promoter activity for GUS reporter gene expression. Table 2 provides the results of this experiment and shows that each of SEQ ID NOS: 1-5 demonstrate promoter activity. The 3' end of each of these fragments is at nucleotide 1643 but each is progressively shorter in length than SEQ ID NO: 1, as shown in attached Table 2 of U.S. Serial No. 10/703,091 on pages 41 and 42 of

U.S. Serial No.: 10/702,319

the specification. Also see attached Figures 1-5 from U.S. Serial No. 10/703,091 (Attachment B), which is marked-up to show that each of the successively shorter fragments is within SEQ ID NO: 1, which as noted above, is within SEQ ID NO: 113. Thus, this data shows that other fragments of SEQ ID NOS: 113, which are shorter than SEQ ID NO: 12 have promoter activity as evidenced by the expression of GUS, preferentially in vascular cambium, xylem, and/or phloem tissues of the transformed plants. With regard to providing a longer promoter containing sequence demonstrating promoter activity, nucleotides 1-1643 of SEQ ID NO: 113 shows promoter activity in Figures 3 and 4 of the present specification.

Applicants submit that the claims now recite a promoter region contained in a specific sequence *E. grandis* cOMT gene (SEQ ID NO: 113) or other specific sequences (SEQ ID NOS: 12 and 60 and other specific nucleotides of SEQ ID NO: 113) that are disclosed in the present application. The specification also provides examples and guidance for making and using polynucleotides, which are all well known to one of skill in the art and which are described in the specification, for example see Examples 1-3 and Figures 3-5. A person of skill in the art could readily identify sequences in SEQ ID NO: 113 using the method described in Example 1, and then test the isolated sequences for promoter activity following the guidance of the present application by testing the sequence in the assays provided in Example 2 in cell culture and in Example 3 in plants. Applicants submit that they have met the standard for determining compliance with the written description requirement which is to convey to a person skilled in that art that the inventor had possession at the time of filing the patent application. See MPEP 2163.02.

The Examiner additionally comments that with regard to claim 2, that the specification fails to disclose any sequence that is hybrizable, having 75%-90% homology with SEQ ID NO: 113, SEQ ID NO: 12 or nucleotides 1019-1643 or which is a 20-600-mer of the above sequences which has vascular specific promoter activity. Again, applicants wish to point out that SEQ ID 113, SEQ ID NO: 12, SEQ ID NO: 60 or specific nucleotides of SEQ ID NO: 113 are recited in the claims and these sequences are disclosed in Figures 1 and

<sup>1</sup> SEQ ID NOS: 1-5 are 534, 485, 306, 293 and 119 nucleotides long, respectively. All end at nucleotide 1643 of SEQ ID NO: 113.

2 or in the sequence listing. Claim 2(c) and 2(d) recite that the sequence has 75% or 90% identity with the recited sequences in (a). Claim 2(e) requires hybridization under stringent conditions which is defined in the specification in the paragraph bridging pages 11 and 12 and on page 19, line 17 to page 20, line 13. Claim 2(f) recites the 20-600-mer of (a) or (d). Again applicants which to point out that the specific sequences are recited in (a), applicants submit that one skilled in the art can make or identify the sequences in claim 2 utilizing the sequences and methods disclosed in the present specification. Thus, a person skilled in the art reading the present specification has the sequences to which percent identity, hybridizability and fragments are determined and assays to identify sequences that possess recited promoter function as in claim 4. Additionally, the examples provide sufficient written description to allow these sequences to be identified and selected by skilled persons in the art.

In view of the clarifying claim amendments and the arguments presented above, it is requested that this rejection be withdrawn to rejected claims 1-11.

### III. Rejections under 35 U.S.C. § 112, second paragraph

Claims 2, 4-8, 10 and 11 are rejected as allegedly being indefinite for the recitation of certain phrases and lack of antecedent basis. Applicants have amended the claims 2 and 4 to make it clear that nucleotides 1019-1643 contained in SEQ ID NO: 113 as supported by the description of Figure 2 on page 5. The word "recited' has been deleted from the claims to further clarify the invention and the dependency of claim 11 has been amended to correct the antecedent basis issue. In view of these amendments to further clarify the invention, it is requested that these rejection be withdrawn.

### IV. Rejection under 35 U.S.C. § 102

Claim 2 is rejected under 35 U.S.C. § 102 as allegedly being anticipated by the 162 nucleotide sequence disclosed in the De Melis *et al.* (AF168777, disclosed on August 19, 1999) because the Examiner alleges that the sequence is the same as a portion of SEQ ID NO: 113. Applicants have located the matching stretch of nucleotides in SEQ ID NO: 113 as shown in Figure 2 of the present invention, which begins at nucleotide 2499 and ends at 2661. This 162 nucleotide portion of SEQ ID NO: 113 is the same sequence that is in SEQ ID NO:

Atty. Dkt. No.: ARBG-004/07US U.S. Serial No.: 10/702,319

60, nucleotides 1524 to 1687, which contains 2096 nucleotides. As explained in the priority section I., above, SEQ ID NO: 60 has a priority date of July 30, 1999 because provisional patent application, U.S. Serial No.: 60/146,591, filed on July 30, 1999, discloses SEQ ID NO: 60. Therefore, because the disclosure of this 162 nucleotide sequence in a priority chain document predates De Melis *et al.*, applicants request that this document be withdraw because it is not prior art against pending claim 2.

### V. Conclusion

Applicants submit that the pending set of claims are in condition for allowance and that the withdrawn claims should be rejoined with the allowable claims according to the *Ochiai* guidelines. The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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47338 v1/DC

## ATTACHMENT A

### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:

J.Phillips et al.

Title:

Vascular-Preferred Promoter Sequences and Uses Thereof

Appl. No.:

Unknown

Filing Date:

11/07/2003

Examiner:

Unknown

Art Unit:

Unknown

### UTILITY PATENT APPLICATION TRANSMITTAL

Mail Stop PATENT APPLICATION Commissioner for Patents PO Box 1450 Alexandria, Virginia 22313-1450

Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(b) is the nonprovisional utility patent application of:

Jonathan PHILLIPS 94 Abbotts Way Remuera Auckland New Zealand

Clare EAGLETON 26C Olberon Street Parnell Auckland New Zealand

### Enclosed are:

- [X] Specification, Claim(s), and Abstract (60 pages).
- [X] Formal drawings (10 sheets) (Figs. 1-10).
- [X] Application Data Sheet (37 CFR 1.76).

The filing fee is calculated below:

	Claims as Filed		Included in Basic Fee		Extra Claims		Rate		Fee Totals
Basic Fee							\$770.00		\$770.00
Total Claims:	31	-	20	=	11	X	\$18.00	=	\$198.00
Independents:	8	_	3		5	x	\$86.00	-	\$430.00
If any Multiple Dependent Claim(s) present: + \$290.00						=	\$0.00		
Surcharge under 37 CFR 1.16(e) for late filing of Executed  + \$130.00						=	\$130.00		
	J					S	UBTOTAL:	=	\$130.00
Small Entity Fees Apply (subtract ½ of above):					=	\$0.00			
		TOTAL FILING FEE:							\$1,528.00

- A check in the amount of \$0.00 to cover the filing fee is enclosed.
- [X] The required filing fees are not enclosed but will be submitted in response to the Notice to File Missing Parts of Application.
- [] The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date III

FOLEY & LARDNER

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Attorney for Applicant

Registration No. 35,792

## VASCULAR-PREFERRED PROMOTER SEQUENCES AND USES THEREOF

### CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional No. 60/425,087, filed November 8, 2002.

#### FIELD OF INVENTION

[0002] The present invention relates to the regulation of polynucleotide transcription and/or expression. In particular, this invention relates to polynucleotide regulatory sequences isolated from *Eucalyptus grandis* that are capable of initiating and promoting the transcription of polynucleotides in plant cells undergoing xylogenesis. Constructs and methods for using the inventive regulatory sequences for modifying transcription of endogenous and/or heterologous polynucleotides also are included in the invention.

### **BACKGROUND OF THE INVENTION**

[0003] Lignin is one of the major products of the phenylpropanoid pathway, and is one of the most abundant organic molecules in the biosphere (Crawford, (1981) Lignin Biodegradation and Transformation, New York: John Wiley and Sons). During lignin biosynthesis, caffeic acid is believed to be O-methylated by caffeic acid/5-hydroxyferulic acid O-methyltransferase (COMT) to form ferulic acid, a direct precursor of lignin. COMT is also believed to play a role in the final hydroxylation reaction of the general phenylpropanoid pathway, in which it O-methylates 5-hydroxyferulate. This dual specificity of COMT has been confirmed by the cloning of the COMT gene, and expression of the protein in *E. coli* (Bugos et al., *Plant Mol. Biol.* 17, 1203, (1991); Gowri et al., (1991) *Plant Physiol.*, 97, 7, (1991)). The expression pattern of the COMT gene and the evidence from mutations of the COMT gene are consistent with this role in lignification (Marita et al., J. Chem. Soc., Perkin

descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional.

[0148] Using the "Genome Walker" kit (Clontech, Palo Alto, CA) and gene-specific primers designed from the plant polynucleotide sequence, 5'UTR sequences containing the putative promoter of the *E. grandis* COMT gene were isolated from genomic DNA and were extended by further sequencing. The nucleotide sequence of the isolated COMT polynucleotide comprising the promoter region and coding region was identified and disclosed in U.S. Provisional Application No. 60/425,087, herein incorporated by reference.

### Example 2: Functional Analysis of *Eucalyptus grandis* Caffeic acid 3-O-Methyl Transferase Promoter

[0149] The xylem specific *Eucalyptus grandis* Caffeic acid 3-O-methyl transferase (COMT) promoter has been dissected into seven fragments (534, 485, 306, 293,119, 99, and 66 bp) based on the location of putative cis-elements that appear in the isolated 1.7kb promoter 5' fragment. These were designed based on the position of putative vascular specific and PAL/AC rich cis-elements in the promoter (GXXXXGTTG; *Plant Molecular Biology* 37: 977-988 1998 and CCATAAACCCC; *Plant Journal* 22: 289-301 2000). The nucleotide sequence of each promoter deletion is disclosed in below in Figures 1-7 (SEQ ID NO: 1-7).

[0150] Table 1 displays each promoter sequence and its homology, as determined by EMBL (EST and genomic) database on May 27, 2003.

Title 1

SEQ ID NO:	Sequence	EMBL Homology	E value
	Length (bp)		
1	534	E.gunnii OMT	5e-05
2	485	E.gunnii OMT	4e-05
. 3	306	E.gunnii OMT	3e-05

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293	E.gunnii OMT	2e-05
119	E.gunnii OMT	9e-06
99	E.gunnii OMT	7e-06
66	E.gunnii OMT	4e-06
	119	119 E.gunnii OMT 99 E.gunnii OMT

### EXAMPLE 3: Methodology to Determine the Tissue Specificity of a Promoter

[0151] Following the identification and cloning of a promoter by the procedure outlined above, the promoter, or fragment thereof, is operably linked with a reporter gene to determine those tissue types in which the promoter is active. To this end, a construct containing the promoter first is transformed into *Agrobacterium tumefaciens* by electroporation. Briefly,  $40~\mu l$  of diluted AgL-l competent cells are placed on ice and are contacted with about 10~ng of pART27 vector containing the promoter sequence. Electroporation is conducted at the following parameters:

Resistance = 129 ohm

Charging voltage = 1.44 kV

Field strength = 14.4 kV/cm

Pulse duration = 5.0 ms

[0152] Following electroporation, 400  $\mu$ l of YEP liquid media is added and the cells are allowed to recover for one hour at room temperature. Cells then are centrifuged at 6000 rpm for 3 min and are resuspended in ~50  $\mu$ l YEP. Cell samples are spread over the surface of a YEP Kan50 / Rif50 plate, sealed with parafilm, and incubated at 29°C for 2 days for colony growth.

[0153] Wild type Arabidopsis thaliana cv. 'Columbia-0' plants are then transformed with Agrobacterium containing constructs of interest by floral dip infiltration. Briefly, Agrobacterium cultures are centrifuged at  $\sim$ 8600 rcf for 10 min at 20°C and are resuspended to an optical density of  $\sim$ 0.7 – 0.8. Plants are dipped into an infiltration solution containing the Agrobacterium for 5 sec. Plants are drained of excess solution and placed under grow lights in ambient conditions. After 24 hrs, the

plants are misted and maintained for seed production. T<sub>1</sub> seeds are surface sterilized in 5% commercial bleach solution and plated on MS media containing Kanamycin (50 mg/l) and Timentin (250 mg/l) to select for putative transformants.

[0154] Successfully transformed plants are then assayed for the expression of the operably linked reporter gene. Leaf, stem, root and floral regions are immersed in a staining solution (50 mM NaPO<sub>4</sub>, pH 7.2, 0.5% Triton X-100, 1 mM X-Glucuronide, cycloheximide salt (Ducheffa). A vacuum is applied twice for 5 min to infiltrate the tissue with the staining solution. The tissue is then left shaking overnight at 37°C for color development. Tissues are checked at three or four time-points to check stain development, and if samples show early development, a piece of tissue is destained in 70% ethanol. This tissue is then examined for GUS expression using a light microscope and photographed.

### Example 4: In Planta Expression Data

[0155] As described in Example 3, transformed Arabidopsis and N. benthamiana tissues are analyzed for GUS reporter gene expression. To assay GUS expression, leaf, root, and floral materials are immersed in the GUS solution as described in Example 2. A vacuum is applied twice for 5 minutes to infiltrate the tissue with the staining solution and the tissue is then incubated overnight in a shaker at 37°C for color development. Following overnight incubation, the tissue samples are then destained in 70% ethanol and examined under a light microscope for GUS expression. Figure 2 displays the percentage of transformed Arabidopsis and N. benthamiana plants expressing GUS.

[0156] Three months post soil transfer, tissues from N. benthamiana T<sub>1</sub> plants are embedded into paraplast, sectioned with a microtome, and analyzed with a light microscope for GUS expression. The GUS localization and microtome results, as shown in Table 2, demonstrate that the disclosed isolated nucleotide sequences confer reporter gene expression preferentially in vascular cambium, xylem, and/or phloem tissues of transformed Arabidopsis and N. benthamiana plants.

Atty. Docket No.: 044463-0263

Table 2: In planta GUS vascular expression

SEQ ID NO	Size (bp)	No Plants GUS +	No Plants Tested	% GUS Expression	GUS Vascular Localization	Microtome Results
1	534	5	12	41.67	Stem material	Xylem, differentiating Cambial cells
2	485	10	12	83.33	Stem, leaf veins	Cambial cells, primary xylem, xylem rays, pith
3	306	9	12	75.00	Stem	Primary and Secondary Xylem, differentiating Cambial cells, Phloem Fiber cells
4	293	9	10	90.00	Stem, developing lateral roots	Xylem, differentiating cambial cells, base and mid sections of xylem rays
5	119	4	12	33.33	Stem, branch points	Xylem
6	99	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested
7	66	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested

[0157] N. benthamiana tissue samples are placed in a vial containing fixative solution (100% Ethanol, Glacial acetic acid, 37% Formaldehyde, mQH<sub>2</sub>0) and a vacuum is applied twice for 15 minutes. The samples are incubated in the fixative solution for two hours to ensure tissue infiltration. Following tissue infiltration, a vacuum is reapplied for 15 minutes and the tissues are placed at 4°C overnight.

[0158] Following overnight incubation, the tissue samples are dehydrated through a series of ethanol incubations, wherein all incubations occur at room temperature. The overnight fixative solution is removed from the vial containing the tissue samples and replaced with 50% ethanol. After 30 minutes, the 50% ethanol is decanted and the tissues are incubated in a fresh aliquot of 50% ethanol. After 30 minutes in 50% ethanol, the solution is removed and replaced with 60% ethanol. Following 30 minute incubation, 60% ethanol is replaced with 70% ethanol. The 70% ethanol is decanted

after 30 minutes and replenished with 85% ethanol. Following 30 minute incubation, the 85% ethanol is removed and the samples are incubated overnight in 95% ethanol.

[0159] Following tissue dehydration, the tissues are incubated at room temperature in a series of xylene solutions. The overnight 95% ethanol solution is removed, and the tissues are incubated in 100% ethanol for 30 minutes. After 30 minutes, 100% ethanol is removed and the tissues are suspended in 25% Xylene:75% Ethanol. Following 30 minute incubation, the solution is replaced with 50% Xylene:50% Ethanol. The solution is then decanted and replenished with 75% Xylene:25% Ethanol. After 30 minutes, the tissues are thrice incubated in 100% Xylene for 60 minutes. The tissues are then overnight incubated in a vial containing xylene and 20 paraplast chips.

[0160] To infuse the paraffin, the vials are placed in a 42°C hybridization oven until the paraplast chips dissolve. Throughout the course of 8 hours, a total of 60-80 paraffin chips are added to the vial and allowed to dissolve. The samples are left overnight in a 62 °C hybridization oven. Over the course of the next two days, the paraplast is changed four times, at 12 hour intervals. To embed the tissues, the liquid paraffin is poured into the cassette and the tissues are placed in the proper orientation. The cassette is then placed at 4°C overnight to allow the paraffin to harden.

# EXAMPLE 5: Isolation and Culture of *Zinnia elegans* Mesophyll Cells in Tracheary Element (TE) inducing (FKH) and non-inducing (FK) medium.

[0161] Primary and secondary pair leaves from the Zinnia seedlings were harvested from 8 punnets. Leaves were sterilized in 500 ml of 0.175% sodium hypochlorite solution for 10 minutes. Leaves were then rinsed twice in 500 ml of sterile water. Using 20-30 leaves at a time, leaves were ground in mortar and pestle and 25-30 ml of FK medium. Cells were filtered through the 40  $\mu$ m nylon mesh. A total of 90 ml of mesophyll cells were obtained in this fashion. Cells were pelleted by centrifuging at 200 x g for 2 minutes at 20° C. The pellet was washed once more using equal volume of FK medium. Then the pellet was split in to two equal halves and one half

## ATTACHMENT B

mid Komed Momodo

Inventor(s): Jayesh VORA et al. Appl. No.: Unassigned Atty. Dkt.: 072121-0315 US.SN 10 703,091

Figure 1: Nucleic Acid Sequence of 534 bp COMT Promoter (SEQ ID NO: 1)

SÉQID NO: 2

ATGCGCCATGTTGACAAAAAGGCTGATTAGTATGATCTTGGAGTTGTTGGTGCAA ATTTGCAAGCTGACGATGGCCCCTCAGGGAAATTAAGGCGCCAACCCAGATTGC AAAGAGCACAAAGAGCACGACCCAACCTTTCCTTAACAAGATCATCACCAGATC GGCCAGTAAGGGTAATATTAATTTAACAAATAGCTCTTGTACCGGGAACTCCGTA

 SEQID No: 4

SEQ II

No:3

GAGTTTTCTCTATATTCTGGTTCACCGGTTGGAGTCAATGGCATGCGTGACGAA TGTACATATTGGTGTAGGGTCCAATATTTTGCGGGAGGGTTGGTGAACCGCAAAG TTCCTATATATCGAACCTCCACCACCATACCTCACTTCAATCCCCACCATTTATCC GTTTTATTTCCTCTGCTTTCCTTTGCTCGAGTCTCGCGGAA

SEQ TO NO: 5 and Kelated Intentions
Inventor(s): Jayesh VORA et al.
Appl. No.: Unassigned

Appl. No.: Unassigned Atty. Dkt.: 072121-0315

Figure 2: Nucleic Acid Sequence of 485 bp COMT Promoter (SEQ ID NO: 2)

and izerated intentions

Inventor(s): Jayesh VORA et al.

Appl. No.: Unassigned Atty. Dkt.: 072121-0315

Figure 3: Nucleic Acid Sequence of 306 bp COMT Promoter (SEQ ID NO: 3)

and Related Methods
Inventor(s): Jayesh VORA et al.
Appl. No.: Unassigned

Atty. Dkt.: 072121-0315

### Figure 4: Nucleic Acid Sequence of 293 bp COMT Promoter (SEQ ID NO: 4)

and Related Methods Inventor(s): Jayesh VORA et al.

Appl. No.: Unassigned Atty. Dkt.: 072121-0315

Figure 5: Nucleic Acid Sequence of 119 bp COMT Promoter (SEQ ID NO: 5)

GGAGGGTTGGTGAACCGCAAAGTTCCTATATATCGAACCTCCACCACCATACCTC ACTTCAATCCCCACCACTTTATCCGTTTTATTTCCTCTGCTTTGCTCGAGTCT CGCGGAA